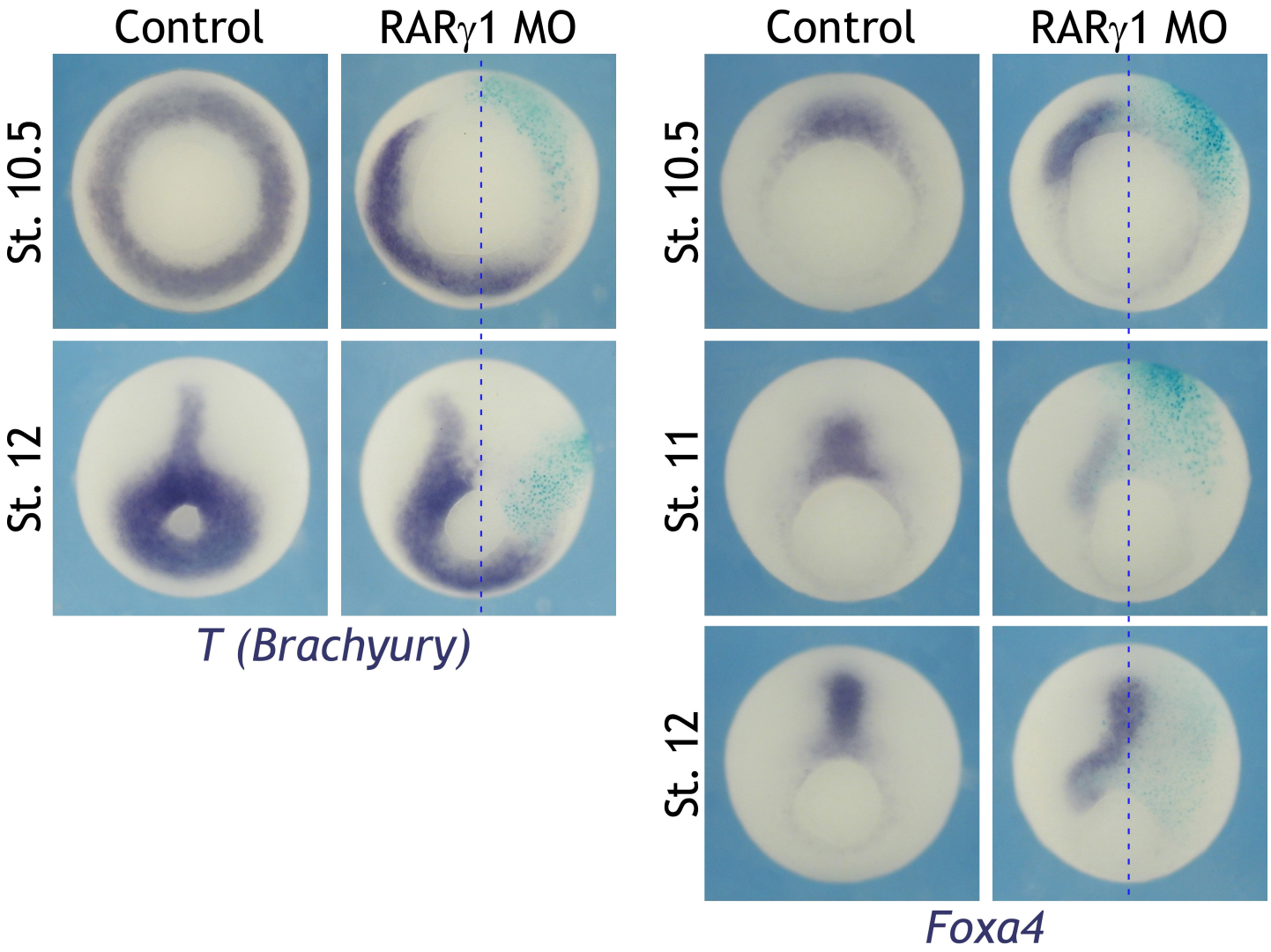
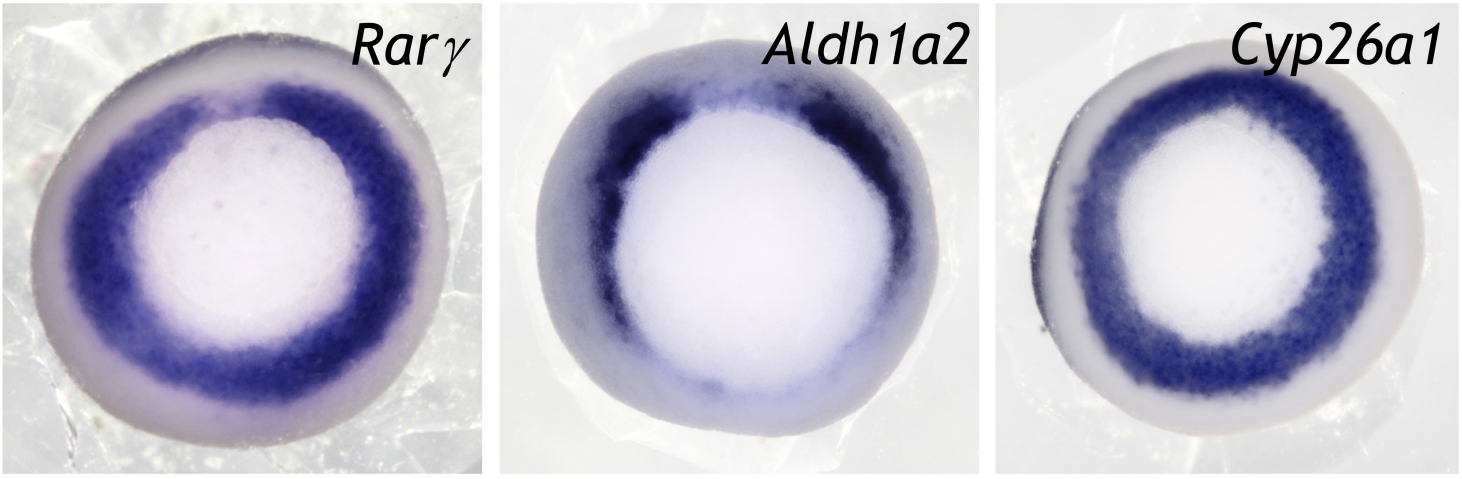


Supplemental Figure S1. RARγ1 is required for the expression of mesoderm markers. Embryos were microinjected unilaterally at the 2-cell or 4-cell stage with 10 ng *Rarγ1*.L/S MO (see Fig S3). Injected side is to the right of the dotted line, and is indicated by the turquoise *β-gal* lineage tracer. *Rarγ1* MO results in the loss of *T (Brachyury)* and *Foxa4*.



Supplemental Figure S2. Whole mount in situ hybridization showing the expression of *Rarγ*, *Aldh1a2*, and *Cyp26a1* at stage 10.5/11, vegetal view, dorsal lip at the top.

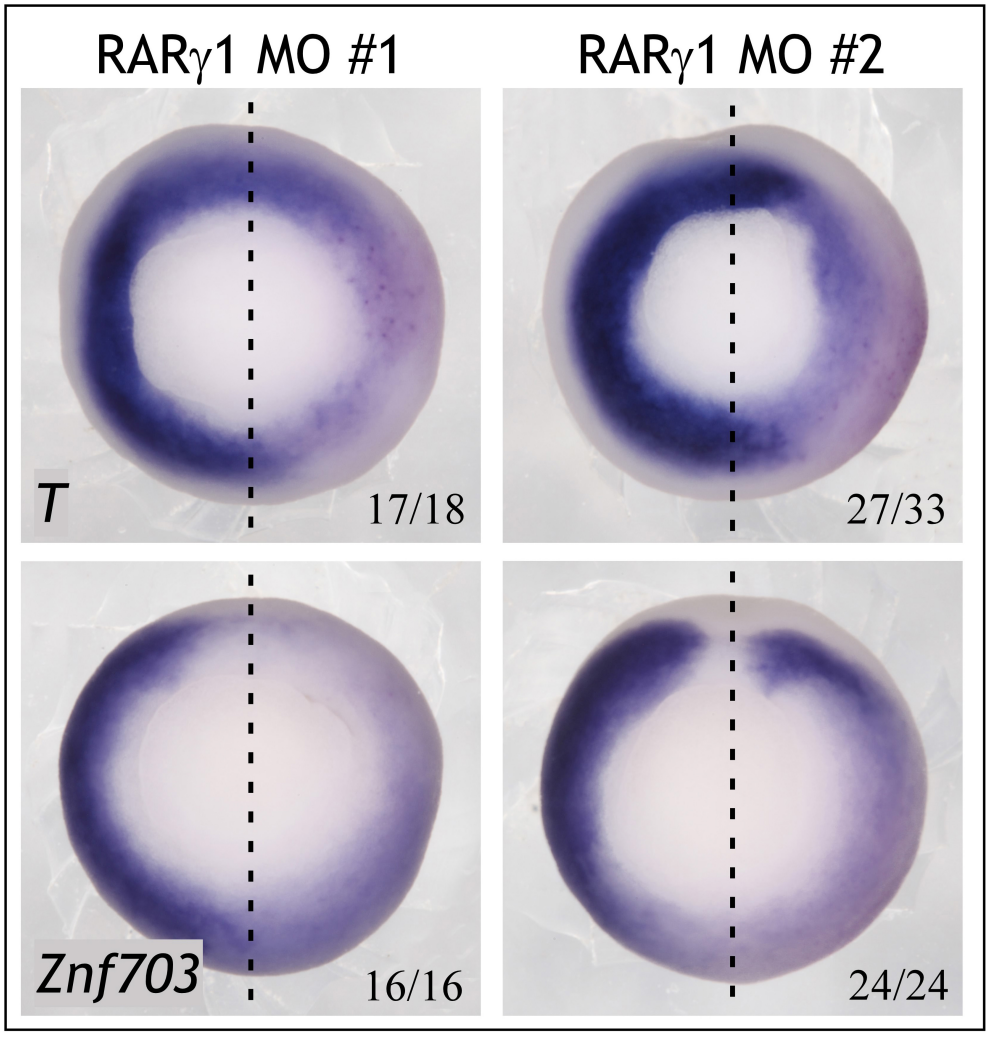
>X. laevis Chromosome 2.S

tcttccacctaaggtttataatggatttatcgctgaataactaaggaagacattg
gtacccaaggaagattccaagacaactactggctagcactaagtaagagactgag
ctagaacaaggca(ATG)GCAAACAGCAGCAAGGAGCGCCTCTGTG

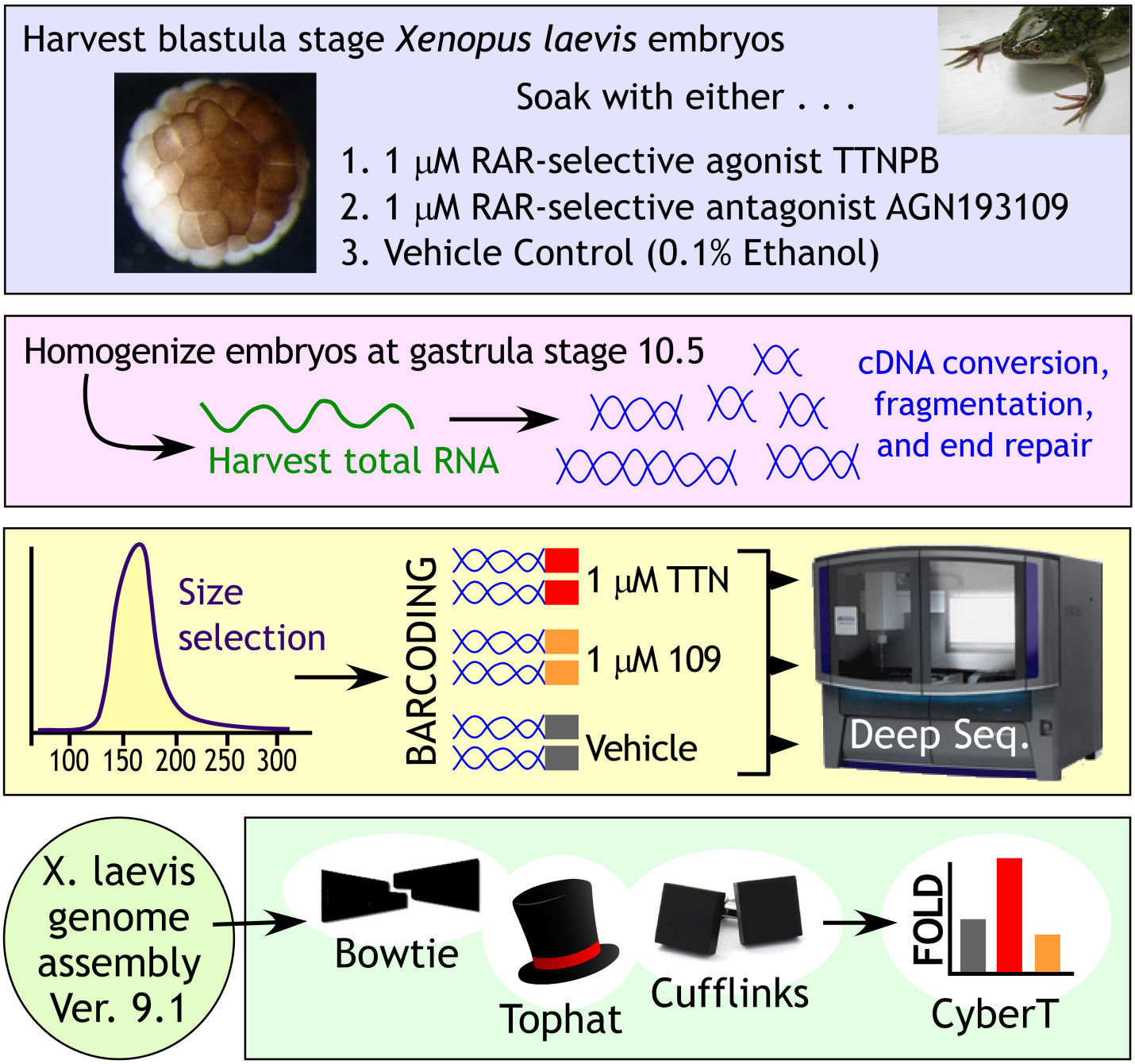
>X. laevis Chromosome 2.L

tcttctacctaaggtgtatcatggatttattgctgaataactaaggaagactttg
gtacccaaagaagattccagga-aactactggctagaactcagtgagagactgag
ggagaacaaggca(ATG)GCAAACAGCAGCAAGGAGCGCCTCTGTG

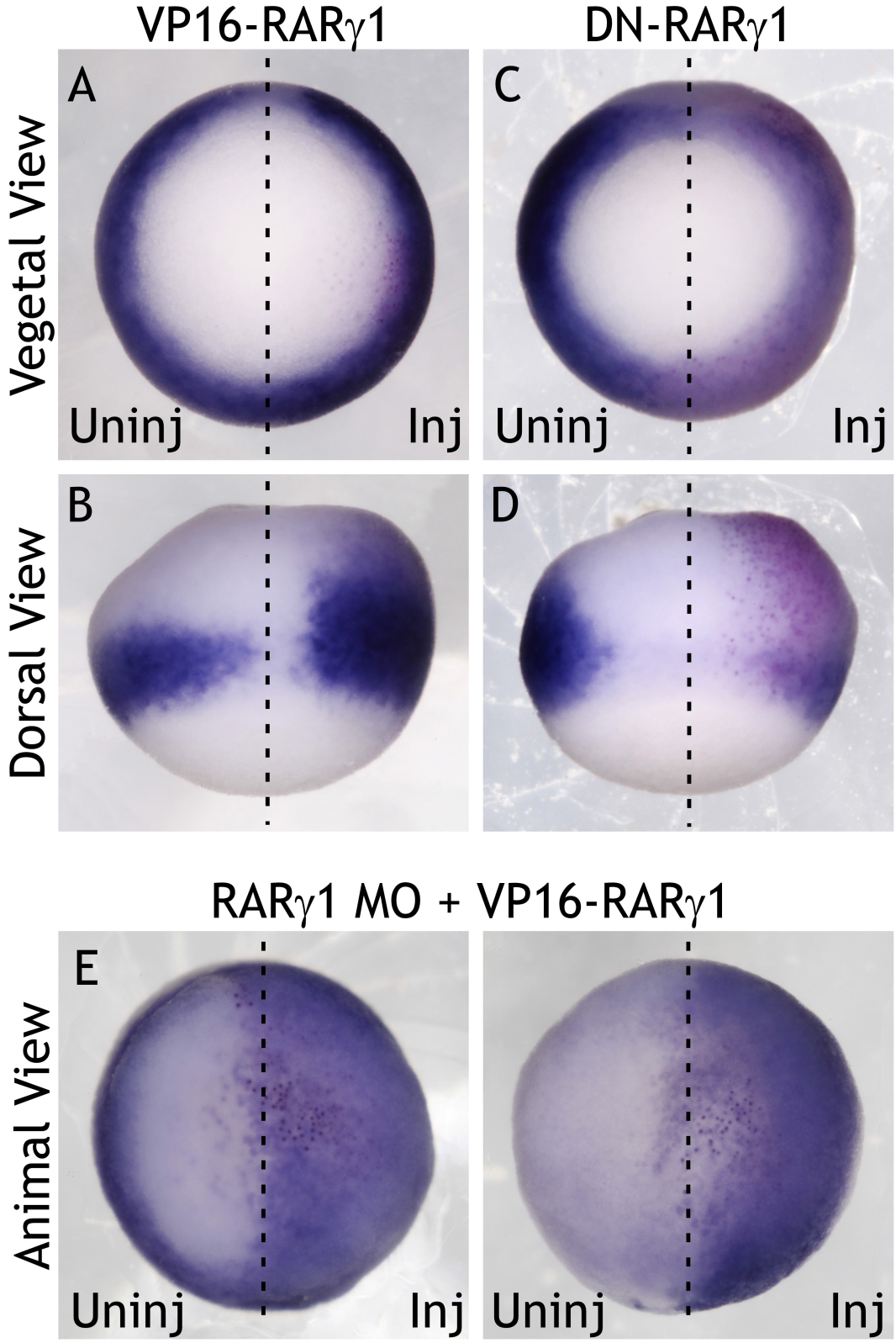
RARγ1 MO #1 RARγ1 MO #2



Supplemental Figure S3. Specificity of RARγ1 MO phenotype. Two different morpholinos were designed to target *Xenopus laevis Rarγ1*. **(Top)** Mapping of MOs to the .S and .L subgenomes (Session, 2016). MO#1 matches nearly perfectly to both .S and .L, whereas MO #2 matches only .S, and will not likely to bind .L. **(Bottom)** Embryos were microinjected unilaterally at the 2-cell or 4-cell stage with 6.6 ng of either *Rarγ1.L/S* MO (#1) or *Rarγ1.S* (#2). Injected side is to the right of the dotted line, and is indicated by the magenta *β-gal* lineage tracer. RARγ1 MO #1 and MO#2 give the same knockdown phenotype on *T* and *Znf703*. Fractions represent the portion of embryos displaying the phenotype.



Supplemental Figure S4. Schematic of the experimental design and RNA-Seq pipeline. Total RNA was isolated from gastrula (stage 10.5) embryos that had been treated at the early blastula (stage 6) with control vehicle (0.1% ethanol), RAR-selective agonist TTNPB (TTN), or RAR-selective antagonist AGN193109 (109). After cDNA synthesis, multiplexed sequencing reactions were run (Aceto et al., 2014). Reads were mapped to *X. laevis* genome v9.1 (Session et al., 2016) using Bowtie, TopHat and Cufflinks algorithms.



Supplemental Figure S5. VP16-*Rarγ1* mRNA expands *Znf703* while DN-*Rarγ1* mRNA diminishes *Znf703*. (A-E) Embryos were injected unilaterally at 2- or 4-cell stage. Injected side is to the right of the dotted line, and is indicated by magenta *ß-gal* lineage tracer. **(A, B, E)** 0.2 ng VP16-*Rarγ1* mRNA expands *Znf703* dorsally and animally. **(C, D)** 2 ng DN-*Rarγ1* mRNA diminishes *Znf703*. Embryos are shown at stage 10.5/11 in vegetal **(A, C)**, dorsal **(B, D)** or animal **(E)** view.

Supplemental Table S1 (1 µM TTNPB) – see Excel sheet
Supplemental Table S2 (1 µM AGN193109) – see Excel sheet

Click here to Download Tables S1 & S2

Supplemental Table S3 (Morpholinos)

MO	Sequence (5'→3')
Rarα2.L	ATC CAA AGG AAG GTG AGT GTG TGT G
Rarα2.S	CTG AAA TCC AAA CTG ACC ATA GAG T
Rarγ1.L/S	GCT GTT TGC CAT TGC CTT GTT CTA
Rarγ1.S	CTA GCC AGT AGT TGT CTT GGA ATC T

Supplemental Table S4 (Probe Design)

Probes with T7 Adapters

Primer	Sequence (5'→3')
Aldh1a2_Probe_For	ACCCTTGAATCTCTAAACAGTGGC
Aldh1a2_Probe_Rev	taatacgactcactatagggAATCTCTTCTCTGGCAATCCGCA
Cyp26a1_Probe_For	GCAGGTTCCTCCAAGTGAAGC
Cyp26a1_Probe_Rev	taatacgactcactatagggCCGCAGAGTCTCCTTAATGACAC
T_Probe_For	GAACGTACAGTACCGGGTGGA
T_Probe_Rev	taatacgactcactatagggTGGTGTGATGGCACTGTTACTC
Gdf3_Probe_For	CTCAGTCTTTCCGTCTCCTTCAC
Gdf3_Probe_Rev	taatacgactcactatagggCAACCACACTCATCCACTACCA
Wnt8_Probe_For	CAACTCTTCTGATCTTCTGCCCA
Wnt8_Probe_Rev	taatacgactcactatagggGTGATTGCCAATATCCCGAAACTC
Skida1_Probe_For	CTGGAGTCGGGCTATGAAGTGG
Skida1_Probe_Rev	taatacgactcactatagggTCCTCCTGTGCCTGTAAGTGG
Kielin_Probe_For	ATTCTGTTGCCACTTCTCTTCTCC
Kielin_Probe_Rev	taatacgactcactatagggATCTCTTCATCCTCCATTTGACGC
Kremen2_Probe_For	TGTTGGTGGAGATGCTGTGG
Kremen2_Probe_Rev	taatacgactcactatagggTCTGGAATCTGGAAGATGTGGA
Nkx6.2_Probe_For	GCCGAGATGAAGACCACTCTG
Nkx6.2_Probe_Rev	taatacgactcactatagggCTCCTCGTTGTCTGAACTCTCC
Mamdc2_Probe_For	ACAGGAAGGGATGTTCTTTGATGC
Mamdc2_Probe_Rev	taatacgactcactatagggTGGCTTTCTGTCCAAACACCA
Znf703_Probe_For	AGCTGAATTCTGTGACCTCCAG
Znf703_Probe_Rev	taatacgactcactatagggCATAAAGCCGTAGGTGTACAAGG
Znf503_Probe_For	CCACTGGGTTCTGGAAGTCG
Znf503_Probe_Rev	taatacgactcactatagggTTTATAGGGTGACACAGGTGC
Dhh_Probe_For	TGCCTAATGTGCCAGAGAAGACTC
Dhh_Probe_Rev	taatacgactcactatagggACAGATGATTGGGTGTAACAAGGA
Btg2_Probe_For	CTCCCGAAAGTCAGCAAGACAC
Btg2_Probe_Rev	taatacgactcactatagggAGACACAAATTGAACCGTCCTCTC
Hapln3_Probe_For	CTTGCCGAATTTGCTGTGATTCTC
Hapln3_Probe_Rev	taatacgactcactatagggGTTCTCCGTAGCTTCTAACACCA
Fstl1_Probe_For	GAGCCCAAGAGCAAGTCTAAGG
Fstl1_Probe_Rev	taatacgactcactatagggCAGATGGTTGGAAGTCGGGA

Supplemental Table S5 (QPCR)

Primer	Sequence (5'→3')
F (*Histone H4*):	GAT AAC ATC CAG GGC ATC AC
R (*Histone H4*):	TAA CCT CCG AAT CCG TAC AG